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THE MASTICATORY HABITS[1]

An Analysis of the Number of Chews When Consuming Food

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The effectiveness of chewing food is a major problem in odontology and, yet, our knowledge of chewing is extremely deficient. Few investigations have been carried out on the efficacy of mastication.

Christiansen (1922) was the first scientist who worked out a method for measuring the efficacy of chewing by persons with full prostheses. He let the individuals in the experiments chew bits of almonds and coconuts and afterwards he sorted out the chews into 4 fractions by straining under sluicing water. The amount of each fraction was determined by weighing after drying. Other authors, including Balters (1928), Gelman (1932) and Ascher (1938), have also used Christiansen's method with slight alterations. They have examined the efficacy of mastication in subjects with full prostheses and in a few persons with their natural dentitions. The investigations were made on a few selected persons and the resulting data can hardly give any definite conception of the masticatory efficiency of the average individual.

Using Christiansen's principles as a basis, the author, in 1942, worked out a method for determining the efficacy of mastication. Standardized bits of gelatin hardened by formalin were used as material for the mastication test. The consistency of this material was similar to cooked veal gristle or hardboiled liver. After chewing the test material, a special apparatus was used for sorting into 10 fractions and the number of particles in each fraction was counted. The total surface of the test portions was calculated with the help of special formulae.

The advantage of chewing food is that the surface of the chewed material increases when the mastication is more effective and the digestives have better opportunities to effect the swallowed food. For that reason the author set up a coefficient for the efficacy of mastication by calculating the surface per volume unit in the dissected food.

In order to understand the present analysis it is necessary to recall in short the main conclusions arrived at in a previous work of the author (Dahlberg, 1942) where the test was used:

The diagram at the bottom of *fig. 1* illustrates the efficacy of mastication expressed in the chewing coefficient (surface per volume unit). As expected, the efficacy of chewing is decreased when the teeth deteriorate, but the mean difference between very good and poor biting surfaces is surprisingly small (35%)

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and considerably less than the difference between individuals with corresponding biting surfaces. The difference of biting surfaces in very good and poor teeth is

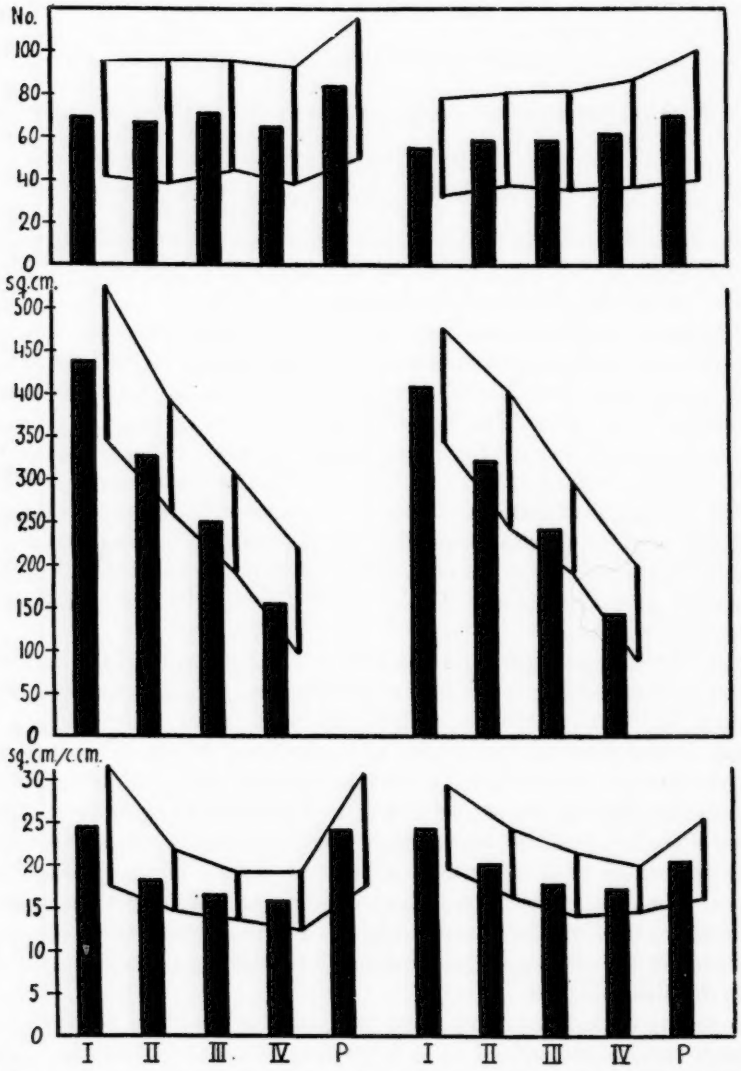

Fig. 1. At the top, the number of chews, on the second line, the occluding surface, at the bottom, mastication coefficients, for men (to the left) and women (to the right) with different sets of natural teeth (groups I–IV) and with prostheses (P). The black columns give the means and the vertical lines the standard deviation. I—extremely good sets of teeth, II—good, III—poor, IV—extremely poor.

65%. (The diagram in the middle.) This means that only a weak or moderate correlation can be found between the surface used for chewing and the efficacy of it. It could be supposed that this unexpectedly small correlation depends on the

compensatory increased number of mastications by those with poor teeth. The top diagram shows, however, that the average number of chews remains constant even in with varying teeth.

It is common knowledge that some persons chew their food carelessly, others carefully and thoroughly. The top diagram (*fig. 1*), showing the number of chews, illustrates this circumstance. The small vertical lines mark the range of variation around the average. The diagram shows that the number of mastications in the test varied around an average of 60–70 chews and that the range of variation could reach down to about 20 and up to about 170 chews. It was possible to show (through repeated tests) that this is a function of habits. These habits may have different bases, e.g., the habit of chewing food to a certain consistency, or the habit of chewing a quantity of food a certain number of times, independent of the consistency reached.

If the degree of crushing (consistency) determines the number of chews by individuals with poor teeth, they will, on the average, compensate the inefficiency of poor teeth by chewing the bolus more often than those with good teeth. If, on the other hand, the way of chewing is habitual and independent of the degree of crushing reached, persons with poor teeth will not chew more often than those with good dentitions. The number of chews will be the same under such circumstances for persons with good or poor sets of teeth. Evidently, that is the case, although this conclusion is surprising. Therefore, it is of interest to complement the analyses on masticatory habits with test substances with experiments on the masticatory habits when consuming real food.

The material for the present investigation was obtained in a home for unmarried mothers and consists of 33 women of 18 to 25 years of age. The material was selected in such a way that about half of the examined persons had good sets of teeth and the rest poor. The classification in regard to the character of the teeth was made after the system used by the author in his previous work.

To compare the number of mastications used by different individuals in consuming a meal it is necessary to standardize the composition of the meal. Furthermore, it is desirable that the test-meal be composed of soft, hard-tough and hard-brittle consistency, approximately in the same relation as in food usually consumed. The food consumed in a Swedish "standardhousehold" includes: about 4.5% hard-tough food, as different sorts of meat, about 5% hard food, as fruits and raw vegetables; about 2% hard-brittle food, as hardbread and biscuits. The bulk of the diet (about 88.5%) normally consumed consists of soft food. In the experiment a standard meal was used weighing a total of 450 gm., composed of 225 gm. potatoes, 100 gm. stewed beans or cabbages, 100 gm. fried bacon or sausage and 25 gm. hardbread. This seems to be fairly representative of the usual dietary.

The investigation has been carried through with double counts, each person having consumed a test-meal on 2 different occasions at about a week's interval. The number of mastications or chews, has been counted, as well as registered with an automatic apparatus, a pedometer rebuilt for the purpose and mounted in an arrangement like earphones. From a spring in the pedometer an elastic ribbon runs around the jaw and is fixed down at the chin. A stretching of the ribbon is

transmitted to the pedometer and registered by its indicator. By regulating the tension in the ribbon the apparatus can be made to register only the more obvious movements of the lower jaw, which are characteristic of mastication. Lesser movements of the lower jaw, as in the case of speaking, are not registered.

To obtain a control, as already mentioned, the number of mastications was ascertained by counting as well as by the automatic apparatus. It turned out that the average number of mastications was higher when registered by the apparatus than by counting. The reason must be that the apparatus registers small and quick movements of the lower jaw which cannot be observed at counting. The difference, however, in the number of mastications arrived at by counting and automatic registering is not significant.

TABLE I

Number of chews when eating a standard meal

	NUMBER	$M \pm \epsilon(M)$	σ
Group I (calculated with apparatus):			
1st test	18	416.89 ± 26.29	111.53
2nd test	18	427.28 ± 26.20	111.15
Group II (calculated by direct observation):			
1st test	15	332.87 ± 20.43	79.13
2nd test	14	366.79 ± 24.43	91.39

$M \pm \epsilon(M)$ = Mean ± standard error of the mean
σ = Standard deviation.

On an average the persons under investigation used 420 ± 26 chews (Table I) when consuming a test-meal. The standard deviation is ±111 mastications, which means that some individuals chewed the meal with 100 mastications only, while others used over 700. This variation in number coincides with the well-known fact, that some individuals chew carelessly, while others chew their food thoroughly.

The total variability, amounting in that case to ±111 chews can be divided into individual and interindividual variation. The interindividual variation is caused by the difference between the separate individuals in the population. The division is done with the help of the formula $o_t = \pm\sqrt{o_i^2\ o_p^2}$ (according to G. Dahlberg, 1926), where o_t stands for the total variation, o_i for the individual variation arrived at by the difference between double counts, and o_p for the interindividual variation arrived at by the formula.

The individual variation amounts to 30 mastications (Table II) and the interindividual to ±105. Thus the division of the total variation shows that the individual range of varation is small and amounts to only 100 chews below or over the average. The interindividual range of variation, on the contrary, forms the bulk of the total variation by varying about 300 mastications below and over the average. If we compare the variability of mastication at testing and consuming food, we find the proportion of individual and interindividual vari-

ation, on the whole, the same. Thus, we find that both at testing and at consuming food, the same individual varies the number of mastications, but this variability is always very small in comparison with the variation among different individuals of the population.

In our exposé, we did not find any appreciable difference in the number of mastications in testing special material and consumption of food. It is, however, quite a different thing to chew a bit of formalin-hardened gelatin and to chew a test-meal. It may therefore be of some interest to see whether the number of mastications is constant when the teeth deteriorate. This was the

TABLE II

The individual variation, σ_i, in the number of chews when eating a standard meal, calculated from the differences between a first and a second test

	NUMBER	σ_i
Group I (calculated with apparatus):		
Diff. between test 1 and 2	18	29.79
Group II (calculated by direct observation):		
Diff. between test 1 and 2	14	30.30

TABLE III

Number of chews when eating a standard meal for persons with good teeth and with poor teeth

	1ST TEST		2ND TEST	
	Number	M ± ϵ(M)	Number	M ±ϵ (M)
Group I: Good teeth	15	376.47 ± 30.43	14	404.21 ± 32.65
Group II: Poor teeth	18	380.56 ± 23.11	18	398.17 ± 21.91
Total:	33	378.70 ± 18.41	32	400.81 ± 18.54

M ± ϵ(M) = Mean ± standard error of the mean.

case in the testing experiments. Table III gives a survey of the average number of mastications with different classifications of the teeth on testing as well as on chewing the standard meal. From the data in Table III, we can see that the average number of mastications with good and poorer sets of teeth is about the same. We find that in the case of moderately hard chewed food the habits of mastication are very constant, which result agrees with that found earlier by the autgor with testing. The habits of mastications do not change when the teeth deteriorate despite the fact that the efficiency of mastication becomes worse. The investigation shows quite clearly, that in the case of moderately hard food, poor teeth are not compensated by better mastication.

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STUDIES IN PERIODONTAL DISEASE

II. Putrefactive Organisms in the Mouth[1]

M. BERG[2] AND L. S. FOSDICK

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In 1939 the osmoscope (1) was adopted for use in measuring the odor intensity of breath (2). It was subsequently shown that periodontal disease was one of the major conditions that was associated with fetor ex ore (3), which suggested that periodontosis might be a putrefactive disease. It was further shown that the production of mal odors was primarily confined to the mouth (4), as a thorough cleansing of the mouth materially reduced the putrefactive odors. In 1943, it was shown that the salivary proteins from individuals suffering from periodontal disease actually putrefied and hydrolyzed much more rapidly than did the salivary proteins from normal individuals (5). This was shown by measuring the rate of odor production and by means of the formol titration. The fact that saliva from individuals with periodontal disease putrefies more rapidly than normal saliva suggests that bacterial action in the two types may be different. In view of this, it was thought interesting to investigate the action of pure strain organisms, isolated from the mouth, in an effort to determine which of the bacteria were capable of causing putrefaction and hydrolysis when allowed to act on human saliva.

It is well known that most of the characteristics of the common mouth organisms have been studied, and it is known which of the organisms are capable of forming products of putrefaction; however, no work has been done under conditions similar to those which are found in the mouth. For this reason, human saliva was used as the test medium for this experiment. The conditions in the mouth are such that aerobic as well as anaerobic conditions are present. Furthermore, there is never a single strain of organism present, which makes possible many types of symbiotic action. For this reason, one series of tests was made using raw saliva. In these experiments large quantities of pure strain organisms were introduced into the raw saliva. The number of organisms used was so large that the number normally present in the saliva was small in comparison. Furthermore, the number of organisms present was sufficiently large so that the enzyme systems of the organisms could act even if no further growth occurred.

In addition to the experiments wherein raw saliva was used, other experiments

[1] This work was done under grants from the Lambert Pharmacal Company and the Colgate-Palmolive-Peet Company. Received for publication, January 22, 1946.

[2] Post Doctorate Fellow in Chemical Research.

on the same samples of saliva were performed with the use of heat sterilized saliva. As heat sterilization usually causes a marked change in pH, other experiments were done in which the heat sterilized saliva was adjusted to pH 6.9 by means of dilute hydrochloric acid. Because blood is sometimes present, samples of all 3 types of saliva containing blood serum were incubated. Each experiment was conducted both aerobically and anaerobically.

EXPERIMENTAL

Approximately 300 cc. of saliva were gathered from several individuals and divided into 3 equal portions. Two of the portions were heat sterilized and the other was used with no further treatment. One of the two heat sterilized portions was used with no further treatment while the other was adjusted to pH 6.9 before use.

One cc. of a heavy saline suspension of the test organism was placed in each of 24 clean sterile test tubes, after which they were centrifuged to settle the organisms and the supernatant fluid was poured off. The tubes containing the organisms were then divided into 3 equal groups and an equal number of clean, dry, sterile test tubes were added to each group. Thus each group contained 16 tubes, half of which contained equal quantities of test organisms. One-half cc. of blood serum was then placed in each of 4 of the clean, dry tubes and into each of 4 of the tubes containing the test organism, and this was repeated with each of the 3 groups. Each group now had 16 tubes, half of which contained blood serum. At this point, 5 cc. of the raw saliva was placed in each of the 16 tubes of one group. The heat sterilized saliva was then placed in the second group of tubes and the heat sterilized saliva that had been adjusted to pH 6.9 was placed in the 16 tubes of the third group. Now, each of the three groups had 4 control tubes containing only saliva, 4 test tubes each of which contained test organism plus saliva, 4 control tubes each of which contained saliva plus blood serum and 4 test tubes which contained saliva, blood serum and test organisms. One of each of the 4 types of tubes was removed from each of the 3 groups and analyzed for pO and degree of hydrolysis, while the others were incubated at 37.5° C. At the end of 1 hour and 3 hours a tube of each type was also removed for a pO determination; and at the end of 24 hours the pO and degree of hydrolysis were determined for the 12 tubes remaining.

The above process was repeated with each of 17 organisms, both under aerobic and anerobic incubation. Furthermore, at least 2 trials under each condition were made in order to secure checks. It was found in many cases, particularly when raw saliva was used, that the addition of the massive quantities of the test organism caused an increase in the rate of putrefaction. It was recognized that this increase might be due to bacterial enzymes or might be due to the addition of more protein, thus furnishing more substrate for putrefaction. For this reason, in those cases where a stimulation of putrefaction was observed, another experiment was performed in which the saliva was incubated with heat sterilized test organisms. This would furnish the equivalent amount of protein (approximately 5 mg. of dried organisms), but all the bacterial enzymes would be

TABLE I

Aerobic incubation

BACTERIA	SERUM	RAW SALIVA					STERILE UNADJUSTED SALIVA					STERILE ADJUSTED SALIVA				
		pO				Hydrolysis	pO				Hydrolysis	pO				Hydrolysis
		F	1h	3h	24h	24h	F	1h	3h	24h	24h	F	1h	3h	24h	24h
—	−	1	3	5	++6	1.38	0	1	1	0	0	1	1	1	1	0
Yeast A	−	1	3	6	++6	1.41	1	1	1	2	.19	1	1	2	1	.07
—	+	1	2	5	+++6	1.6	0	1	1	1	.28	1	1	0	1	0
Yeast A	+	1	4	6	+++6	2.42	1	1	1	1	.5662	1	0	1	1	.075
—	−	1	3	5	++6	1.38	1	1	1	1	0	1	1	1	1	0
Yeast B	−	1	4	5	+++6	1.47	1	1	1	2	.275	1	1	1	2	.03
—	+	1	2	5	+++6	1.60	1	1	1	1	.435	1	1	0	1	0
Yeast B	+	1	3	6	+++6	2.42	1	1	1	2	.367	1	0	1	1	.05
—	−	1	3	5	++6	1.58	1	1	1	1	.093	1	1	1	1	0
Sarcina lutea	−	1	5	6	+++6	2.18	1	1	3	2	.443	1	1	2	1	.285
—	+	1	2	5	+++6	1.60	1	1	1	1	.292	1	1	0	1	0
Sarcina lutea	+	1	+6	+++6	+++6	2.14	1	1	4	1	.377	1	2	2	1	.22
—	−	1	3	4	++6	1.73	0	1	0	0	0	1	1	1	1	0
Str. viridans	−	1	3	4	+++6	1.5	0	1	1	1	0	1	0	1	0	.01
—	+	1	2	5	+++6	2.13	0	0	0	1	.11	1	1	1	0	.024
Str. viridans	+	1	2	5	+++6	2.2	0	0	1	0	.09	1	0	1	0	.024
—	−	1	3	5	++6	1.38	0	1	0	0	0	1	1	1	1	0
B. subtilis	−	1	6	+++6	+++6	1.52	0	1	1	1	.05	1	1	1	1	.04
—	+	1	2	5	+++6	1.6	0	0	0	1	.11	1	1	1	0	.024
B. subtilis	+	1	5	+++6	+++6	3.08	0	1	1	1	.165	1	1	1	1	.045
—	−	1	3	5	++6	1.38	0	1	0	0	0	1	1	1	1	0
G. tetragena	−	1	4	+6	+++6	1.61	0	1	1	1	0	1	1	1	1	0
—	+	1	2	5	+++6	1.60	0	0	0	1	.11	1	1	1	0	.005
G. tetragena	+	1	3	5	+++6	2.33	0	1	1	1	.07	1	1	1	1	0
—	−	1	3	4	++6	1.73	0	1	0	0	0	1	1	1	1	0
L. acid.	−	1	2	5	++6	1.63	0	1	0	1	.025	1	1	1	1	0
—	+	1	2	5	+++6	2.13	0	0	0	1	.11	1	1	1	0	.0075
L. acid.	+	1	1	4	++6	2.81	0	1	0	1	.17	1	1	1	2	.09
—	−	1	3	5	+++6	1.38	1	1	1	1	0	1	1	1	1	.02
Pr. vulgaris	−	1	5	+++6	+++6	1.54	1	4	4	3	.14	1	1	4	1	.03
—	+	1	2	5	+++6	1.60	1	1	1	0	0	1	1	1	1	.145
Pr. vulgaris	+	1	6	+++6	+++6	2.34	1	5	6	4	.065	1	2	3	4	.20
—	−	1	3	5	++6	1.38	1	1	1	1	0	1	1	1	1	.02
Ps. aeruginosa	−	1	+6	+++6	+++6	1.63	1	4	3	2	0	1	1	3	+6	.105
—	+	1	2	5	+++6	1.6	1	1	1	1	0	1	1	1	1	.125
Ps. aeruginosa	+	1	+6	+++6	+++6	2.68	1	4	3	1	.015	1	1	2	6	.11

TABLE I—*Continued*

BACTERIA	SERUM	RAW SALIVA pO F	RAW SALIVA pO 1h	RAW SALIVA pO 3h	RAW SALIVA pO 24h	RAW SALIVA Hydrolysis 24h	STERILE UNADJUSTED SALIVA pO F	STERILE UNADJUSTED SALIVA pO 1h	STERILE UNADJUSTED SALIVA pO 3h	STERILE UNADJUSTED SALIVA pO 24h	STERILE UNADJUSTED SALIVA Hydrolysis 24h	STERILE ADJUSTED SALIVA pO F	STERILE ADJUSTED SALIVA pO 1h	STERILE ADJUSTED SALIVA pO 3h	STERILE ADJUSTED SALIVA pO 24h	STERILE ADJUSTED SALIVA Hydrolysis 24h
—	−	0	1	4	++6	1.58	0	0	0	0	.045	1	2	2	0	.025
S. albus	−	0	1	+6	++6	1.95	0	1	2	1	.05	1	2	2	1	.13
—	+	0	1	5	++6	2.01	0	0	0	1	.162	1	1	1	1	.155
S. albus	+	0	1	++6	++6	2.56	0	0	1	1	.247	1	2	2	2	.195
—	−	0	1	4	++6	1.58	1	1	1	0	.187	1	2	2	0	.025
S. citreus	−	0	2	++6	++6	1.85	1	2	1	3	.135	1	2	2	2	.075
—	+	0	1	5	++6	2.01	1	1	1	1	.557	1	1	1	1	.155
S. citreus	+	0	2	++6	++6	3.14	1	2	3	4	.428	1	1	2	1	.16
—	−	0	1	4	++6	1.58	0	1	1	0	.035	1	2	2	0	.024
S. aureus	−	0	2	++6	++6	1.84	0	1	2	2	.045	1	4	3	1	.05
—	+	0	1	5	++6	2.01	0	1	1	1	.137	1	1	1	1	.155
S. aureus	+	0	1	++6	++6	3.18	0	1	2	2	.301	1	1	3	1	.185
—	−	1	3	4	++6	1.63	0	1	0	0	0	0	1	1	0	0
Actinomyces	−	1	2	5	+++6	1.39	0	1	1	0	.032	0	1	1	1	0
—	+	1	2	5	+++6	2.13	0	0	0	1	.11	0	0	1	1	.07
Actinomyces	+	1	2	4	+++6	2.47	0	1	1	0	.085	0	1	1	2	.06
—	−	1	3	5	++6	1.38	0	1	0	0	0	1	1	1	1	0
M. albicans	−	1	4	6	+++6	1.51	0	1	1	1	0	1	1	1	1	0
—	+	1	2	5	+++6	1.6	0	0	0	1	.11	1	1	1	1	0
M. albicans	+	1	4	+6	+++6	2.62	0	1	0	0	.07	1	1	1	2	0
—	−	1	3	5	++6	1.38	1	1	1	1	0	1	1	1	1	0
A. aerogenes	−	1	4	+++6	+++6	1.59	1	2	3	1	0	1	4	+6	5	0
—	+	1	2	5	+++6	1.6	1	1	1	1	0	1	1	1	1	0
A. aerogenes	+	1	4	+++6	+++6	2.1	1	2	3	2	0	1	3	5	5	.14
—	−	1	3	5	+++6	1.38	1	1	1	1	0	0	1	1	0	0
Leptothrichia	−	1	4	++6	+++6	1.52	1	3	3	3	.06	0	3	4	2	0
—	+	1	2	5	+++6	1.60	1	1	1	1	0	0	0	1	1	.07
Leptothrichia	+	1	5	+6	+++6	1.84	1	1	3	3	.01	0	3	4	1	.07
—	−	1	3	5	++6	1.38	0	0	0	0	.10	1	1	1	0	0
N. catarrhalis	−	1	3	6	+++6	1.57	0	1	2	4	0	1	2	3	2	0
—	+	1	2	5	+++6	1.6	0	0	0	1	.095	1	1	1	0	.08
N. catarrhalis	+	1	3	6	+++6	2.13	0	1	1	3	.01	1	1	1	1	.08

inactivated. In no case did the addition of the heat-killed organisms increase the rate of putrefaction.

In every case the organisms were originally isolated from the saliva or tooth scrapings and identified by the usual methods. It should be noted that although

TABLE II

Anaerobic incubation

BACTERIA	SERUM	RAW SALIVA					STERILE UNADJUSTED SALIVA					STERILE ADJUSTED SALIVA				
		pO				Hydrolysis	pO				Hydrolysis	pO				Hydrolysis
		F	1h	3h	24h	24h	F	1h	3h	24h	24h	F	1h	3h	24h	24h
—	−	1	3	3	+6	1.60	1	1	1	1	0	1	1	1	1	.03
S. citreus	−	1	+6	5	++6	1.50	1	1	1	2	0	1	1	1	1	.155
—	+	1	1	3	+6	1.82	1	1	1	1	0	1	1	1	1	.01
S. citreus	+	1	4	4	+++6	2.59	1	1	1	1	.035	1	1	1	1	.22
—	−	1	3	3	+6	1.605	1	1	1	1	0	1	1	1	1	.025
S. aureus	−	1	6	6	++6	1.585	1	1	1	1	0	1	1	1	5	.17
—	+	1	1	3	+6	1.775	1	1	1	1	0	1	1	1	1	.01
S. aureus	+	1	+6	6	+++6	3.065	1	1	1	1	.055	1	1	1	6	.21
—	−	1	3	3	+6	1.605	1	1	1	1	0	1	1	1	1	.03
S. albus	−	1	5	4	++6	1.70	1	1	1	1	0	1	1	1	1	.115
—	+	1	1	3	+6	1.775	1	1	1	1	0	1	1	1	1	.01
S. albus	+	1	5	6	+++6	2.56	1	1	1	1	.015	1	1	1	2	.045
—	−	1	1	4	++6	1.24	1	1	1	1	0	0	0	1	1	0
Yeast A	−	1	1	3	5	1.02	1	1	1	1	.015	0	0	2	1	0
—	+	1	1	1	5	1.55	1	1	1	1	0	0	1	2	1	0
Yeast A	+	1	2	2	+++6	1.38	1	2	1	1	.035	0	1	1	1	.02
—	−	1	2	4	5	1.31	0	0	0	1	.06	0	1	2	2	.075
Yeast B	−	1	2	3	+6	1.16	0	1	0	1	0	0	1	1	4	.08
—	+	1	2	3	+6	1.81	0	0	1	1	0	0	0	1	1	.22
Yeast B	+	1	2	3	6	1.86	0	0	1	2	0	0	1	2	3	.09
—	−	1	2	4	5	1.31	0	0	0	1	.065	0	1	2	3	.075
N. catarrhalis	−	1	1	4	6	1.11	0	1	1	1	.06	0	3	6	3	.305
—	+	1	2	3	+6	1.81	0	0	1	0	0	0	0	1	1	.22
N. catarrhalis	+	1	2	3	+6	1.9	0	0	1	4	.095	0	2	6	1	.16
—	−	1	2	4	5	1.31	0	0	0	1	.06	0	1	2	2	.075
Ps. aeruginosa	−	1	2	4	++6	1.07	0	1	0	5	.695	0	2	6	4	.835
—	+	1	2	3	+6	1.81	0	0	1	0	0	0	0	1	1	.22
Ps. aeruginosa	+	1	2	3	++6	1.43	0	1	1	++6	.86	0	4	++6	4	.80
—	−	1	1	4	++6	1.24	1	1	1	0	0	0	0	1	1	0 ..
Pr. vulgaris	−	1	1	3	5	1.42	1	1	1	3	.095	0	1	1	1	.04
—	+	1	1	1	5	1.55	1	0	1	1	0	0	1	2	1	0
Pr. vulgaris	+	1	1	3	+++6	1.63	1	1	1	5	0	0	1	2	1	.015
—	−	1	1	4	++6	1.24	1	1	1	0	0	0	0	1	1	0
G. tetragena	−	1	1	3	+6	1.38	1	1	2	1	0	0	0	1	1	0
—	+	1	1	1	5	1.55	1	0	1	1	0	0	1	2	1	0
G. tetragena	+	1	2	4	+++6	1.82	1	1	1	1	.02	0	1	1	1	0

TABLE II—*Continued*

BACTERIA	SERUM	RAW SALIVA					STERILE UNADJUSTED SALIVA					STERILE ADJUSTED SALIVA				
		pO				Hydrolysis	pO				Hydrolysis	pO				Hydrolysis
		F	1h	3h	24h	24h	F	1h	3h	24h	24h	F	1h	3h	24h	24h
—	−	1	1	3	6	1.50	0	2	1	1	0	1	1	1	1	0
Sarcina lutea	−	1	1	3	5	1.83	0	1	1	1	.015	1	1	1	2	.135
—	+	1	2	1	5	2.43	0	1	1	1	0	1	1	1	1	.02
Sarcina lutea	+	1	1	2	+6	2.9	0	1	1	1	.155	1	1	1	2	.41
—	−	1	1	3	6	1.49	0	2	1	1	0	1	1	1	1	0
A. aerogenes	−	1	1	4	3	1.44	0	2	2	4	0	1	1	1	2	.015
—	+	1	2	1	5	2.43	0	1	1	1	0	1	1	1	1	.02
A. aerogenes	+	1	2	3	++6	2.30	0	2	3	4	.03	1	1	1	3	.135
—	−	1	1	2	5	1.2	1	1	1	1	0	0	0	0	1	.05
M. albicans	−	1	2	5	4	1.27	1	1	1	1	0	0	0	0	1	.01
—	+	1	2	5	6	1.54	1	1	1	1	0	0	0	1	1	.01
M. albicans	+	1	1	3	5	2.87	1	1	1	1	0	0	0	0	2	.08
—	−	1	1	3	6	1.49	0	2	1	1	0	1	1	1	1	0
B. subtilis	−	1	2	3	6	1.2	0	1	1	1	.085	1	1	1	4	.26
—	+	1	2	1	5	2.43	0	1	1	1	0	1	1	1	1	.02
B. subtilis	+	1	1	4	4	2.32	0	1	1	1	.135	1	1	2	++6	.19
—	−	1	1	2	5	1.2	1	1	1	1	.06	0	0	0	1	.05
Actinomyces	−	1	1	4	++6	1.34	1	1	1	1	0	0	0	0	2	.075
—	+	1	2	5	6	2.54	1	1	1	1	0	0	0	1	1	.01
Actinomyces	+	1	1	4	++6	2.86	1	1	1	1	0	0	0	1	2	.085
—	−	1	1	2	5	1.2	1	1	1	1	0	0	0	0	1	.05
Leptothrichia	−	1	1	2	2	1.36	1	1	1	1	0	0	1	1	2	.12
—	+	1	1	1	4	1.06	1	1	1	1	0	0	0	1	1	.01
Leptothrichia	+	1	1	2	++6	1.84	1	1	1	1	.015	0	1	1	2	.12
—	−	1	3	5	++6	1.57	1	1	1	2	0	1	1	1	1	0
Str. viridans	−	1	2	5	+++6	1.15	1	1	1	2	0	1	1	1	2	.065
—	+	1	4	6	+++6	2.11	1	1	1	2	0	1	1	1	2	.01
Str. viridans	+	1	1	3	+++6	1.77	1	1	1	2	0	1	1	1	2	.165
—	−	1	3	5	+++6	1.57	1	1	1	2	0	1	1	1	1	0
L. acid.	−	1	3	6	+++6	1.39	1	1	1	2	0	1	1	1	1	.015
—	+	1	4	6	+++6	2.11	1	1	1	2	0	1	1	1	2	.01
L. acid.	+	1	4	+6	+++6	2.31	1	1	1	2	0	1	1	1	2	.01

all of the organisms used were isolated from the mouth, many were not necessarily constant inhabitants of all mouths but may be only transients. After obtaining the organisms, they were grown on large agar surfaces prepared in flat 1-liter bottles. They were then harvested and suspended in isotonic salt solutions. The Tables I and II contain the average results of at least 2 trials with each organism.

DISCUSSION

The procedures used in this experiment, although they are not of the orthodox variety, should in some respects yield results similar to those one would expect to obtain in the mouth. There are regions in the mouth in which anaerobic conditions prevail as well as regions of the mouth in which aerobic conditions prevail. Furthermore, the saliva always contains large quantities and large numbers of various organisms, so that under these conditions one would expect many symbiotic effects.

When saliva was incubated either aerobically or anaerobically, odors were produced very rapidly, so at the end of 3 hours' incubation the saliva was usually quite putrid and at the end of 24 hours was always very putrid. Furthermore, hydrolysis of the salivary proteins proceeds quite rapidly under these conditions, so that at the end of 24 hours in all saliva there is a large number of protein fragments. In view of this, it is quite remarkable that foul mouth odors are not encountered more frequently.

There are many laboratory procedures that could be used to determine the degradation of the protein, such as analyses for indole, skatole and amino acid. For this purpose the formol titration was used because it is simple, accurate and requires small samples. This procedure, however, only determines the change in carboxy groups which are liberated by hydrolysis of the protein and thus does not measure the amount of amino acid or polypeptide that has been destroyed or altered by other action. In view of the limitation of the test, the correlation of the results with odor concentration is quite significant.

The addition of bacteria to the raw saliva would mean nothing unless the organisms were added in sufficient quantities so that the organisms normally present would be in the minority. If the addition of large quantities of organisms stimulates the production of odors and the hydrolysis of the protein over that which is obtained in the controls, it is obvious that the organisms added either caused the formation of the excess odors and excess hydrolysis or provided certain enzyme systems which made the other organisms present capable of producing t[1] se changes. Because blood is sometimes present in cases of periodontal disease and because blood is known to contain certain factors which accelerate the growth of bacteria, it is possible that the presence of blood would cause the saliva to be a more favorable medium and hence may stimulate odor production and hydrolysis. That this is true is obvious from some of the experiments.

Although it is known that many of the organisms can produce putrefaction on an ideal synthetic medium, they have never been investigated on a plain saliva medium. Insofar as it is extremely difficult, if not impossible, to obtain sterile saliva without altering the pH of the saliva, the experiment wherein unadjusted saliva was used should not be a true criterium of bacterial action in the mouth. Nevertheless, it will give some information concerning whether or not each organism can grow in this medium and can tolerate the reaction obtained by each such sterilization of the saliva.

The sterile adjusted saliva should produce results which would indicate whether or not any single strain of organism can produce odors and putrefaction without the addition of extraneous substances.

When one reviews the results it is obvious that practically all of the organisms tried, with the exception of *L. acidophilus*, *Sarcina lutea*, yeast and *S. viridans*, accelerate the normal putrefaction of raw saliva in the absence of blood and under aerobic incubation. In the presence of blood serum, there is usually an increase in the rate of odor production and an increase in the degree of hydrolysis over those samples in which no blood was used. In a few cases, however, such as with *L. acidophilus*, the presence of blood serum did not accelerate the putrefaction.

Under anaerobic conditions, the rate of odor production seems to be of about the same order as under aerobic incubation, with the exception that at the 24 hour interval the amount of odor produced was not as intense as when the same saliva was incubated aerobically. The degree of hydrolysis, however, was usually of the same order of magnitude. Under these conditions and in the absence of blood serum, all of the organisms stimulated odor production with the exception of yeast, *N. catarrhalis*, *Pr. vulgaris*, *G. tetragena*, *Sarcina lutea*, *A. aerogenes*, and Leptothrichia. In the presence of blood serum only the elliptical yeast, *N. catarrhalis*, *M. albicans* and *B. subtilis* did not stimulate odor production and hydrolysis.

In general, the heat sterilized saliva, whether adjusted or not, did not support the action of the oral bacteria nearly as well as the raw saliva. This may indicate that there are heat labile substances present in raw saliva that are conducive to bacterial growth, or that the bacteria depend quite heavily on each other to produce growth substances.

With heat sterilized, unadjusted saliva incubated under aerobic conditions and in the absence of blood serum, only *N. catarrhalis*, *Ps. aeruginosa*, *Pr. vulgaris*, *A. aerogenes*, *Sarcina lutea*, *S. citreus* and Leptothrichia produced appreciable odors and hydrolysis. The presence of blood under the conditions produced similar results.

Under anaerobic conditions, only *N. catarrhalis*, *Ps. aeruginosa*, *Pr. vulgaris* and *A. aerogenes* produced hydrolysis and putrefactive odors. The presence of blood serum did not effect the results.

The heat sterilized, pH adjusted saliva supported the action of more organisms than did the unadjusted only under anaerobic conditions. Under aerobic conitions, *Pr. vulgaris*, *Ps. aeruginosa*, *S. viridans*, *A. aerogenes*, Leptothrichia and *M. catarrhalis* produced putrefaction. The presence of blood serum under these conditions produced no changes in the results.

Under anaerobic conditions, the heat sterilized, adjusted saliva supported the action of elliptical yeast, *N. catarrhalis*, *Ps. aeruginosa*, *B. subtilis*, *S. aureus* and *A. aerogenes*.

SUMMARY

The rate of putrefaction of saliva, under the influence of 17 organisms isolated from the mouth and under varying conditions, was studied. It was found that practically all of the organisms studied may play some rôle in the putrefaction of salivary proteins. It is further evident that under the conditions of the experiment, no single type of organism is capable of producing putrefaction as rapidly as the mixtures that are normally present in the mouth.

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THE EDITOR'S VIEWPOINT

We met one individual at the Twenty-fourth General Meeting who had already received his February issue. It was our hope that the February number would be in the hands of our readers by the time of the Association's meeting which was March 16 and 17, not 15 and 16 as erroneously announced in this JOURNAL. With the help of our authors and printers we expect to have this April issue to our subscribers in April.

All sessions of the Twenty-fourth General Meeting were well attended and there were many lively discussions. Unfortunately the spirit of the discussions is only available to those present but there is much to be gained from the "Proceedings" which will be published in the June number of the JOURNAL.

From the Naval Dental School, Commander Parke sends us an important paper on an important clinical subject, the effectiveness of instrument sterilization (p. 89). Perhaps dentists are aware of the problems of instrument sterilization more than any other single group. It is important to find a feasible method that will kill pathogens without unduly damaging instruments. Dr. Parke has carefully tested one of the methods advocated for instrument sterilization and presents his data. We believe that such studies are essential rather than loose banter. Appleton lists 9 influencing factors on the antibacterial action of chemicals in solution: the nature of the organisms, variations in resistance within a bacterial species, the nature of the chemical, the solvent, the concentration, the proportion of culture to chemical, the temperature at which the test is performed, the time of exposure and the nature of the medium. He suggests that any and all procedures should be tested for their effectiveness under the conditions obtaining in each individual office (Appleton, J. L. T., *Bacterial Infection*, Phila., Lea and Febiger: 1944). Commander Parke has reported on the value of hot oil bath sterilization for naval installations and has compared its effectiveness with others.

This month we again present a paper from overseas. Dr. Dahlberg's study on chewing efficiency and the oral condition (p. 67) suggests similar problems such as the relationship of masticatory effectiveness to periodontal diseases, to acrylic and procelain teeth, and to ceratin specific dietary deficiencies. Dahlberg's monograph, *The Masticatory Effect*, is suggested for further reading on this subject.

The Schamps (p. 101) have reported in a field 'contributory to stomatology.' Their results in a study of pain threshold should be of value to those investigating certain phases of physiologic effects in anesthesia.

Material prepared by students to meet a thesis requirement below the graduate level is usually in the nature of a literature review. Drs. Mandel and Sarkady (p. 95) went beyond this stage and carried out a laboratory investigation on the use of a "tracer substance." The authors recognize the limitations of the present report but present a method for possible use for investigators with greater facilities and more time.

Periodontal disease may be studied through an evaluation of the putrefactive organisms in the oral cavity. Using this approach, Drs. Berg and Fosdick (p. 73) found that mixtures of oral organisms were more capable of producing putrefaction than any single organism.

Dr. Weisberger carries on his study of growth factors for *L. acidophilus* (p. 83). He reports that these organisms appear to require nitrogenous substances for maximum growth and that these are not available in concentrated whole saliva. We would like to see the comparative growth value of saliva from patients with high and low caries activity.

H. B. G. R.

WHOLE SALIVA AS A SOURCE OF CERTAIN GROWTH FACTORS FOR AN ORAL STRAIN OF LACTOBACILLUS[1]

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Recently (1) we have reported experiments in which *Lactobacillus acidophilus* strain 333, isolated from human saliva, grew successfully and produced acid maximally in a synthetic medium made up from casein hydrolysate, tryptophane, mono- and di-potassium phosphates,[2] thiamin hydrochloride, nicotinic acid, calcium pantothenate and dextrose. In the present report experiments are described in which it was found that whole saliva could replace some but not all constituents of this synthetic medium.

EXPERIMENTAL

Test organism. The isolation and the culturing of strain 333 of *Lactobacillus acidophilus* used in these experiments was carried out in accordance with procedures outlined in an earlier paper (1).

Preparation of media. A stock solution of all constituents of the basal medium was routinely made up in volumes of 30 ml. It contained the substances listed in Table I and in the quantities stated there. In preparing the single strength complete media for test experiments 3 ml. portions of the stock were used. The concentrations in the stock solutions were worked out to permit a large portion (5 ml.) of saliva to be employed in some of the test experiments and also to provide opportunity to adjust the pH before diluting to the final volume of 10 ml. used in all test experiments. Other stock solutions were prepared from which, in turn one only of the essential constituents was omitted. The concentrations of substances in these solutions having a known deficiency was the same as those in the complete medium. In use these solutions were supplemented by saliva to determine whether that fluid could replace the particular constituent which had been omitted.

Collection and treatment of saliva. In the first experiments saliva collected from subjects taken at random was used. These individuals chewed paraffin to stimulate the secretory activity of the glands. The fluid was used without filtration or preliminary treatment. It was added in quantities of 5 ml. to each of a series of tubes containing 3 ml. of one of the series of stock preparations of basal media. The contents of these tubes were then treated in accordance with the test procedure described below. Data typical of the tests on untreated saliva are given in Table II.

[1] Received for publication February 11, 1946.

[2] Designated in tables as salt mixture A.

Specimens of saliva secreted with the aid of paraffin stimulation were found to contain 0.425–0.455 mg. of amino acid nitrogen and 3.75–3.95 mg. of total nitrogen respectively per 5 ml. In contrast the quantity of casein hydrolysate used in preparing synthetic media for test experiments provided 5.9 mg. of amino acid nitrogen and 11.0 mg. of total nitrogen. It was thought that the saliva as collected might not provide nitrogenous substances in concentrations sufficient to

TABLE I

Concentrations in stock solutions of substances essential for maximum acid production

SUBSTANCES	QUANTITY PER 30 ML.
Casein hydrolysate[a] (Vitamin free)	2.5 ml.
Salt mixture A*	0.5 ml.
d-Glucose[c]	1.0 gm.
Sodium acetate[b]	600.0 mg.
Tryptophane[d]	10.0 mg.
Thiamin hydrochloride[b]	10.0 y
Nicotinic acid[b]	20.0 y
Calcium pantothenate[b]	20.0 y
Distilled water	to 30.0 ml.

Sources of Materials Used in the Media.

[a] S. M. A. Co.

[b] Merk & Co.

[c] Baker Chemical Co.

[d] Eastman Kodak Co.

* Salt Mixture A.

K_2HPO_4	5.0 gm.
KH_2PO_4	5.0 gm.
Distilled Water	to 50 ml.

TABLE II

Substitution of whole saliva for various essential nutrients in a synthetic medium

SUBSTANCE OMITTED*	SALIVA ADDED PER 10 ML. OF MEDIA	AMOUNT OF ACID PRODUCED
	ml.	*ml.*
Casein Hydrolysate	5.0	0.00
Tryptophane	5.0	0.02
Nicotinic Acid	5.0	0.00
Thiamin Hydrochloride	5.0	0.00
Calcium Pantothenate	5.0	0.13
Salt Mixture A†	5.0	4.85
Inoculum (sterility control)	5.0	0.00
None (growth control)	None	4.99
None (inhibition control)	5.0	4.97

* d-Glucose present in all experiments.

† K_2HPO_4 and KH_2PO_4.

promote growth of the organism. Therefore a second series of experiments was performed. In these the saliva from a number of individuals was collected, pooled and concentrated sevenfold. This increased the concentration of nitrogenous substances to approximately that which the casein hydrolysate provided when it was used in the test solutions. Details regarding the experiments of this series follow.

Three lots of saliva were collected and concentrated in vacuo at 45° C. The first lot contained 525 ml. and was provided by 5 individuals. The mixed, unfiltered specimen was concentrated to 75 ml. At that stage amino acid nitrogen as determined by the Van Slyke nitrous acid method (2) was 3.1 mg. per 5 ml. while the total nitrogen by Kjeldahl (3) was 26.8 mg. per 5 ml. The concentrate thus prepared was used in tests as a substitute for the hydrolyzed casein of the synthetic media. On another day the same quantity of saliva was collected

TABLE III

Substitution of whole saliva concentrate for various essential nutrients in a synthetic basic medium

SUBSTANCE OMITTED*	SALIVA CONCENTRATE ADDED PER 10 ML. OF MEDIA	AMOUNT OF ACID PRODUCED
	ml.	*ml.*
Casein Hydrolysate	5.0	0.00
Tryptophane	5.0	0.00
Nicotinic Acid	5.0	1.40
Thiamin Hydrochloride	5.0	0.26
Calcium Pantothenate	5.0	0.50
Inoculum (sterility control)	5.0	0.00
None (growth control)	None	4.87
None (inhibition control)	5.0	4.84

* d-Glucose present in all experiments.

from the same 5 persons. It was concentrated similarly and was used as a substitute for tryptophane in the synthetic medium. In a third experiment 10 individuals, including the 5 previously used, contributed 1575 ml. After sevenfold concentration, it was used as replacement, in turn, for each of the vitamins previously found essential. The data obtained when the saliva concentrates were used in test procedures are presented in Table III.

Test procedures. An experiment in which tryptophane was omitted from the basal media may be used to illustrate our procedures. The stock solution of the basal medium was made up without this amino acid. Three ml. of this preparation was placed in a series of tubes and 5 ml. of untreated saliva concentrate was added to each tube. The tubes were adjusted to a pH of 6.8, made up to a volume of 10 ml. with distilled water, mixed and then autoclaved at 10 pounds pressure for 10 minutes.

The tubes containing the test materials were inoculated with 0.1 ml. of a

suspension of the test organism handled as described previously (1). The tubes were incubated for 72 hours at 37.5° C. Then the contents of each tube in turn were rinsed into 50 ml. beakers with the aid of about 5 ml. of distilled water. The acid produced by the micro organism was titrated to a pH of 6.8 with 0.1 N sodium hydroxide using a Coleman pH Electrometer.

Three control tests were set up as a part of each experiment. In one control the addition of inoculum was omitted in order to demonstrate the sterility of the saliva concentrate. In a second control some saliva concentrate was added to synthetic medium which was complete in all essentials in order to test for any inhibitory action of the saliva concentrate upon the test organism. In the third control the organism was added to the complete synthetic medium in order to demonstrate that experimental conditions (temperature, time, etc.) were adequate for maximal acid production when all necessary factors were present.

RESULTS

Data obtained in our experiments with whole, unconcentrated saliva are presented in Table II. The results observed when concentrated specimens were used are recorded in Table III.

These observations indicate that whole saliva as secreted under the stimulus of paraffin chewing is capable of providing or replacing the potassium phosphates contained in a synthetic mixture of substances essential for maximum acid production in the presence of *Lactobacillus acidophilus*. These findings also indicate that the amino acids and other forms of nitrogen provided by casein hydrolysate and added tryptophane are either not available in whole saliva, or not present in the necessary forms and quantities even in saliva concentrated sevenfold. In this connection the fact that the Hopkins-Cole reaction, which is a test for combined tryptophane, was positive in the whole saliva supports the unavailability hypothesis. On the other hand our data points to the presence of low but definite concentrations of several water soluble vitamins in saliva. Whether these have been excreted through the salivary glands or have accumulated from foods consumed remains to be demonstrated. With regard to the presence of pantothenic acid in human saliva our findings are in agreement with the reports of Hill and Kniesner (4, 6) and of Kniesner, Mann and Spies (5).

SUMMARY

1) Whole human saliva used without any concentration satisfactorily replaced the mineral salt fraction of a synthetic medium capable of supporting growth and maximal acid production of an oral strain of *Lactobacillus acidophilus*.

2) Whole saliva, even when concentrated sevenfold, did not serve as a substitute for casein hydrolysate or for tryptophane of the synthetic medium.

3) Concentrated whole saliva did aid in the production of slight growths and low acid production when substituted in turn for thiamin hydrochloride or calcium pantothenate or nicotinic acid.

4) These findings indicate that the nitrogenous substances essential for maximum acid production with this organism are, either not present in sufficient amounts in whole saliva even when concentrated sevenfold, or are not in a form available for use by the organism.

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STERILIZATION EFFECTIVENESS OF A HOT OIL BATH[1]

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Due to a pressing need for a method of preserving dental handpieces during World War II, an elaborate plan was instituted at one of our largest Naval training centers to investigate and, if possible, to provide a solution to the cause of rapid deterioration of such instruments.

A survey revealed that the universal method used by 350 dental officers at this one activity for the sterilization of dental handpieces was simply that of wiping the instrument with gauze saturated in alcohol. This method was inadequate for the following reasons: (a) Gross debris was not removed from the intricate gears in the handpiece, (b) Proper lubrication after attempted sterilization was not accomplished, (c) The method at best was an effort at partial cleaning but not sterilizing.[2]

A method had to be devised whereby the dental handpieces could be cleaned, lubricated and sterilized in a manner satisfactory to the less energetic, to promote the life of the handpiece and to insure a sterile instrument for each patient. Oil sterilization (Liquid Petrolatum, USP, Heavy, heated to a temperature of 250°F., in a sterilizer) preceded by the application of a solvent solution was the method of choice.[3] The effectiveness of the solvent solution for the removal of gross debris from the gears of the handpiece was soon established. (1) It remained to demonstrate the effectiveness of hot oil as a sterilizing medium. The practicability of sterilizing handpieces by immersion in a hot-oil bath had been shown and vigorously advocated by Appleton as early as 1924. (2) However, it appears that such procedures have been ignored by most practicing dentists.

METHOD AND RESULTS

Dental handpieces were contaminated in the following manner: (a) By operating the handpiece, equipped with a rubber cup, in the mouths of patients for a period of 5 minutes. During this time, the gears of the handpiece were well

[1] "The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large." (Art. 113, USNaval Regulations). Received for publication March 5, 1946.

[2] Investigations conducted at this naval training center disclosed 138 positive bacteriologic findings of 138 tests on handpieces so treated.

[3] The dental handpiece, before being placed in the hot oil bath was operated in a solution of 1 part carbon tetrachloride, 1 part xylol and 2 parts liquid petrolatum, USP. [The xylol increases the solvent action but is not a necessity. Should xylol be omitted, then equal parts of carbon tetrachloride and liquid petrolatum is recommended.] (For bacteriological tests as submitted in this report the solvent solution was omitted.)

inoculated with organisms from the churned saliva as revealed by actual tests conducted. Tests on 493 such contaminated handpieces were recorded. (*Fig. 2*). (b) By immersing and operating handpieces for thirty seconds in pure cultures of known organisms. The results of 729 tests were tabulated using this method. (*Figs. 3* and *4*).

After contamination, the handpieces were completely immersed in liquid petrolatum, USP, (Heavy) at various temperatures for a period of 2 minutes. Upon removal from the oil bath the handpieces were attached to a motor driven

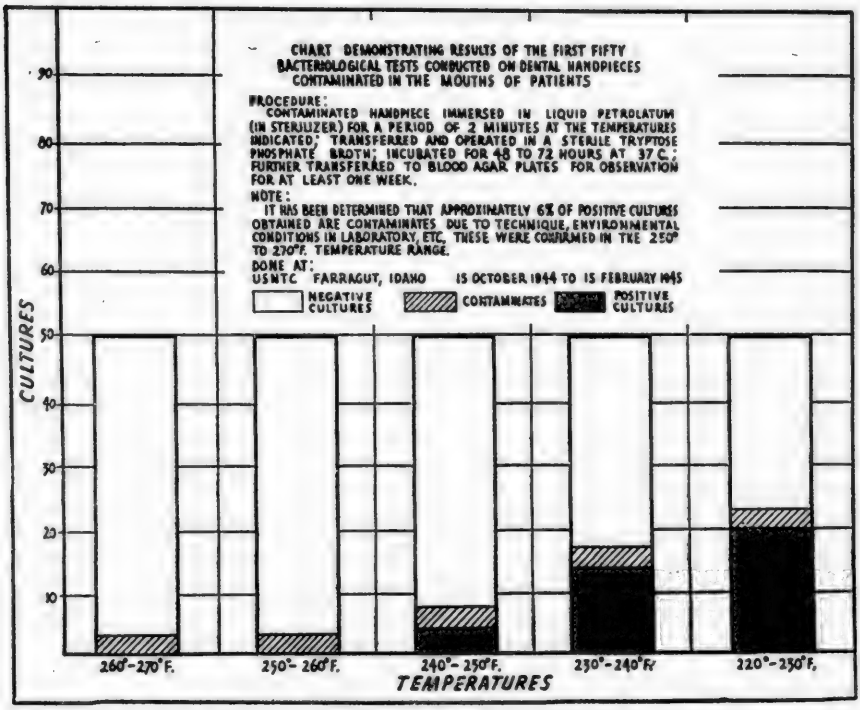
CHART DEMONSTRATING RESULTS OF THE FIRST FIFTY BACTERIOLOGICAL TESTS CONDUCTED ON DENTAL HANDPIECES CONTAMINATED IN THE MOUTHS OF PATIENTS
PROCEDURE:
CONTAMINATED HANDPIECE IMMERSED IN LIQUID PETROLATUM (IN STERILIZER) FOR A PERIOD OF 2 MINUTES AT THE TEMPERATURES INDICATED; TRANSFERRED AND OPERATED IN A STERILE TRYPTOSE PHOSPHATE BROTH; INCUBATED FOR 48 TO 72 HOURS AT 37 C.; FURTHER TRANSFERRED TO BLOOD AGAR PLATES FOR OBSERVATION FOR AT LEAST ONE WEEK.
NOTE:
IT HAS BEEN DETERMINED THAT APPROXIMATELY 6% OF POSITIVE CULTURES OBTAINED ARE CONTAMINATES DUE TO TECHNIQUE, ENVIRONMENTAL CONDITIONS IN LABORATORY, ETC. THESE WERE CONFINED IN THE 250° TO 270°F. TEMPERATURE RANGE.
DONE AT:
USNTC FARRAGUT, IDAHO IS OCTOBER 1944 TO 15 FEBRUARY 1945
NEGATIVE CULTURES
CONTAMINATES
POSITIVE CULTURES
CULTURES
90
80
70
60
50
40
30
20
10
260°-270°F.
250°-260°F.
240°-250°F.
230°-240°F.
220°-230°F.
TEMPERATURES

Fig. 1

unit by means of sterile forceps and operated for 30 seconds in test tubes containing sterile tryptose phosphate broth. The cultures were then incubated at 37° C. for periods of 48 hours to 72 hours. Identification of surviving organisms was established, in the event growth occurred, after subculturing the centrifuged sediment onto blood agar plates. The majority of cultures were observed for a period of 1 week prior to disposal.

A further group of experiments was conducted to establish the thermal death points of common oral pathogenic organisms. The results of these experiments were used to determine the contamination factor during bacteriologic tests on dental handpieces. It was observed that contaminants were introduced in the amount of 6% of the first 50 tests in each temperature range above 250° F. (*Fig. 1*). This was attributed to prevailing laboratory conditions and accidental con-

tamination. As the experiments progressed, the percentage of contaminants was reduced to 2.4% of the total number of tests conducted. (*Fig. 3*).

Staphylococcus aureus was found to be the most resistant of all organisms tested, however this organism was destroyed consistently in 55 seconds at 250° F. (*Fig. 4*). When the death point of the organism was established, 30 tests or more were conducted at that point for confirmation. Thirty consecutive negative reactions was accepted as conclusive evidence of the destruction of the organism. The

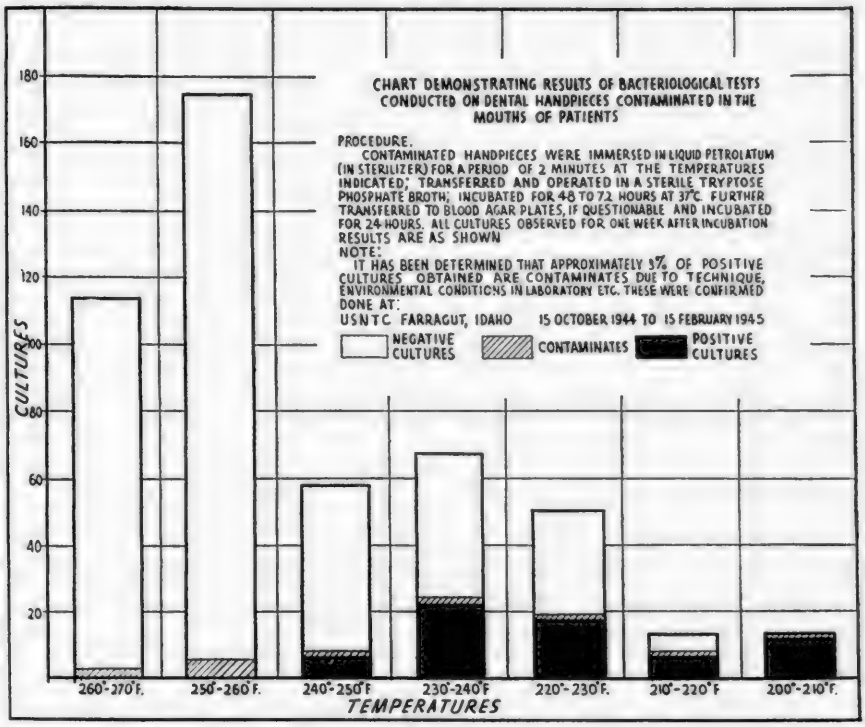
CHART DEMONSTRATING RESULTS OF BACTERIOLOGICAL TESTS CONDUCTED ON DENTAL HANDPIECES CONTAMINATED IN THE MOUTHS OF PATIENTS
PROCEDURE.
CONTAMINATED HANDPIECES WERE IMMERSED IN LIQUID PETROLATUM (IN STERILIZER) FOR A PERIOD OF 2 MINUTES AT THE TEMPERATURES INDICATED; TRANSFERRED AND OPERATED IN A STERILE TRYPTOSE PHOSPHATE BROTH; INCUBATED FOR 48 TO 72 HOURS AT 37°C. FURTHER TRANSFERRED TO BLOOD AGAR PLATES, IF QUESTIONABLE AND INCUBATED FOR 24 HOURS. ALL CULTURES OBSERVED FOR ONE WEEK AFTER INCUBATION RESULTS ARE AS SHOWN
NOTE:
IT HAS BEEN DETERMINED THAT APPROXIMATELY 5% OF POSITIVE CULTURES OBTAINED ARE CONTAMINATES DUE TO TECHNIQUE, ENVIRONMENTAL CONDITIONS IN LABORATORY ETC. THESE WERE CONFIRMED
DONE AT:
USNTC FARRAGUT, IDAHO 15 OCTOBER 1944 TO 15 FEBRUARY 1945
NEGATIVE CULTURES
CONTAMINATES
POSITIVE CULTURES
CULTURES
180
160
140
120
100
80
60
40
20
260°-270°F.
250°-260°F.
240°-250°F
230°-240°F
220°-230°F.
210°-220°F
200°-210°F.
TEMPERATURES

Fig. 2

media used and the technic employed was the same as that in previous experiments. In determining the results shown in *fig. 4*, 252 separate tests were conducted.

All bacteriological tests on dental handpieces were performed within a few seconds to several hours after contamination. For this reason the results may be considered those of tests on wet organisms. No appreciable resistance on the part of the organisms was encountered unless a delay of 8 hours or more intervened between contamination of the handpiece and the bacteriologic test. Since sterilization of the handpiece in hot oil directly after its use on each patient is recommended, then so-called dry organisms are not a major factor in the sterilization of dental handpieces. Dry organisms, however, may become wet organisms by immersing the handpiece for a few seconds in tap water before cleaning

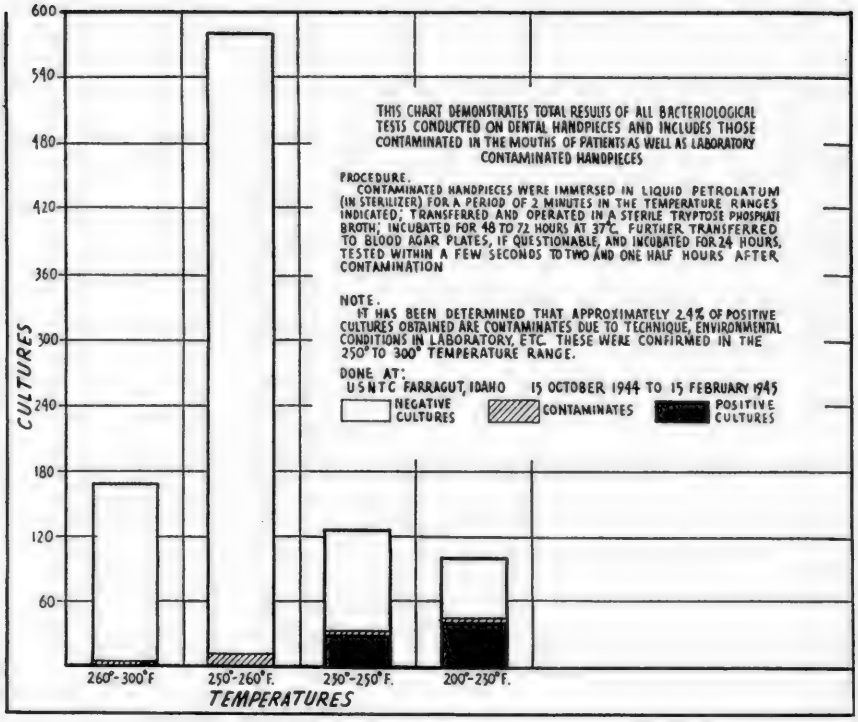
600
540
480
420
360
300
240
180
120
60
CULTURES
THIS CHART DEMONSTRATES TOTAL RESULTS OF ALL BACTERIOLOGICAL TESTS CONDUCTED ON DENTAL HANDPIECES AND INCLUDES THOSE CONTAMINATED IN THE MOUTHS OF PATIENTS AS WELL AS LABORATORY CONTAMINATED HANDPIECES
PROCEDURE.
CONTAMINATED HANDPIECES WERE IMMERSED IN LIQUID PETROLATUM (IN STERILIZER) FOR A PERIOD OF 2 MINUTES IN THE TEMPERATURE RANGES INDICATED; TRANSFERRED AND OPERATED IN A STERILE TRYPTOSE PHOSPHATE BROTH; INCUBATED FOR 48 TO 72 HOURS AT 37°C. FURTHER TRANSFERRED TO BLOOD AGAR PLATES, IF QUESTIONABLE, AND INCUBATED FOR 24 HOURS. TESTED WITHIN A FEW SECONDS TO TWO AND ONE HALF HOURS AFTER CONTAMINATION
NOTE.
IT HAS BEEN DETERMINED THAT APPROXIMATELY 2.4% OF POSITIVE CULTURES OBTAINED ARE CONTAMINATES DUE TO TECHNIQUE, ENVIRONMENTAL CONDITIONS IN LABORATORY, ETC. THESE WERE CONFIRMED IN THE 250° TO 300° TEMPERATURE RANGE.
DONE AT:
USNTC FARRAGUT, IDAHO 15 OCTOBER 1944 TO 15 FEBRUARY 1945
NEGATIVE CULTURES
CONTAMINATES
POSITIVE CULTURES
260°-300°F
250°-260°F.
230°-250°F.
200°-230°F.
TEMPERATURES

Fig. 3

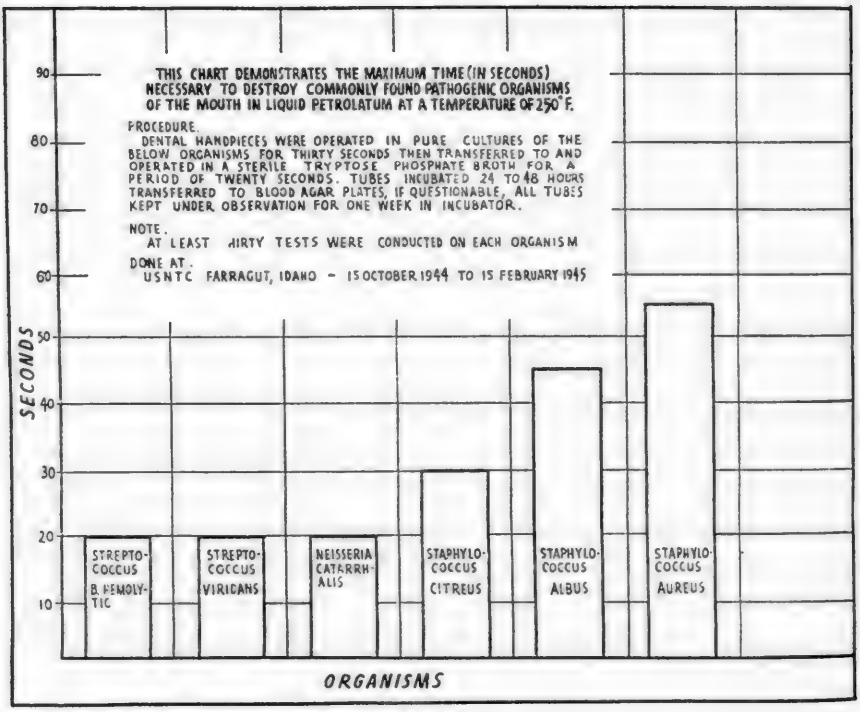
90
80
70
60
50
40
30
20
10
SECONDS
THIS CHART DEMONSTRATES THE MAXIMUM TIME (IN SECONDS) NECESSARY TO DESTROY COMMONLY FOUND PATHOGENIC ORGANISMS OF THE MOUTH IN LIQUID PETROLATUM AT A TEMPERATURE OF 250° F.
PROCEDURE.
DENTAL HANDPIECES WERE OPERATED IN PURE CULTURES OF THE BELOW ORGANISMS FOR THIRTY SECONDS THEN TRANSFERRED TO AND OPERATED IN A STERILE TRYPTOSE PHOSPHATE BROTH FOR A PERIOD OF TWENTY SECONDS. TUBES INCUBATED 24 TO 48 HOURS TRANSFERRED TO BLOOD AGAR PLATES, IF QUESTIONABLE, ALL TUBES KEPT UNDER OBSERVATION FOR ONE WEEK IN INCUBATOR.
NOTE.
AT LEAST THIRTY TESTS WERE CONDUCTED ON EACH ORGANISM
DONE AT.
USNTC FARRAGUT, IDAHO - 15 OCTOBER 1944 TO 15 FEBRUARY 1945
STREPTOCOCCUS B. HEMOLYTIC
STREPTOCOCCUS VIRIDANS
NEISSERIA CATARRHALIS
STAPHYLOCOCCUS CITREUS
STAPHYLOCOCCUS ALBUS
STAPHYLOCOCCUS AUREUS
ORGANISMS

Fig. 4

and sterilizing. The sterilizing time will thus be reduced to that for wet organisms.

Spore forming organisms were considered and tested in conjunction with other bacteriologic tests. It was determined that oil at 250° F. was no more effective than boiling water (20 minutes) in destroying such organisms. In the light of the fact that boiling water has been universally accepted by the professions for sterilization of instruments used in dental and minor surgical operations and it does not destroy spores, then it may be appropriate to regard such organisms as insignificant in oil sterilization procedures.

In all, 1767 bacteriologic tests were performed on dental handpieces using the hot-oil method here described.

SUMMARY

(a) Sterile handpieces were consistently obtained by immersing them after use on each patient in Liquid Petrolatum, USP (Heavy) for a period of 2 minutes at a temperature of 250° Fahrenheit.

(b) Dental handpieces were well lubricated as a result of this method and remained so during operations in the mouth.

(c) Cold sterilization methods were ineffective, time consuming and damaging to the intricate gears in the handpiece.

(d) Boiling water was damaging to the gears in the handpiece and did not remove gross debris.

(e) No presently known method will remove debris such as mucous, tooth filings, pumice, etc., from the inside of the dental handpiece as does a solvent solution followed by the flushing action of hot oil.

(f) No simpler method could be found to clean, sterilize and lubricate such a mechanism so effectively in so short a period of time.

(g) Dental handpieces exposed to 14 months of oil sterilization procedures have resulted in no breakdown in the mechanism of the handpiece.

CONCLUSION

The complete immersion of dental handpieces in liquid petrolatum for a period of 2 minutes at a temperature of 250° F. following operation of the instruments in a suitable solvent solution does produce a degree of sterilization equal to that of boiling water, and, in addition, very materially prolongs the life of the handpiece.

Acknowledgement: The author wishes to gratefully acknowledge the assistance of the following in carrying out the experiments as recorded in this report: (1) Lieutenant H. Bailie (DC) USNR, (2) Lieutenant C. M. Stebner (DC) USNR, (3) Lieutenant Commander A. F. Errington (MC) USNR.

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INTRODUCTION OF SODIUM IODIDE INTO THE CALCIFIED DENTAL TISSUES[1]

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This report deals with one aspect of tooth permeability, namely, the introduction of a foreign substance into the calcified dental tissues. Primarily its purpose is the development of a new method for studying this phenomenon, utilizing sodium iodide as the foreign substance.

Investigation in tooth permeability, especially with regard to enamel, has considered two aspects of the problem—penetration from the pulp outward to the external surface, and from without, inward. The methods employed in these studies included: Various staining solutions applied to the whole tooth in vitro (1), (2). Complex dyes inserted into the pulp and applied to the enamel surface in vivo (3), (4). Introduction of radioactive salts in vitro and in vivo (6, 7, 8, 9, 10).

These procedures have provided considerable data on the subject, but are nevertheless open to criticisms. Results published on the basis of experimentation with extracted teeth can be considered only an indication of the true picture because loss of vitality has been shown to influence permeability (3). Investigators using dyes in direct contact with the teeth, for the most part, have employed complex molecular dyes. Since permeability depends largely on the size and charge of the molecule, results obtained from the use of these dyes cannot be applied to calcium, phosphorous, fluorine, and other small inorganic ions which bathe the teeth in vivo. Neither the use of extracted teeth nor dyes contacting the teeth can be correlated with normal physiological conditions.

The use of radioactive isotopes, while an excellent method of investigation, requires extensive equipment, is a difficult procedure, and does not clearly demonstrate the distribution of the introduced substance. It has nevertheless contributed much information concerning the degree of tooth permeability in vivo. Wasserman and Blayney (5, 6), Volker and Sognnaes (7, 10), and Barnum and Armstrong (9) have demonstrated that radioactive phosphorous injected intraperitoneally or intravenously finds its way into the dentin and enamel of the teeth. The dentin, as might be expected, takes up considerably more phosphorous than the enamel. All investigators agree that the phosphorous which finds its way into the dentin does so through the blood. As far as the enamel is

[1] Presented at the New York Section of the International Association for Dental Research, June 7, 1945. (J. D. Res. 24: 5, 267, 1945). Received for publication January 14, 1946.

[2] Thesis submitted in partial fulfillment of requirments for the degree of Doctor of Dental Surgery at the School of Dental and Oral Surgery, Columbia University.

concerned, there is considerable dispute as to whether the phosphorous comes from the pulp, the saliva, or both. Barnum and Armstrong (9) stated that some phosphorous comes into the enamel from the saliva, but that most of it enters via the blood stream. Volker and Sognnaes (10), by dividing the enamel into 3 parts based on density, found that the more dense third or surface enamel had the greatest concentration of radioactive phosphorous. They concluded from this that the phosphorous present in the enamel was salivary in origin. This was not a demonstration of actual penetration into the enamel, however, but more probably of adsorption. The phosphorous may have been attracted to the enamel surface and held there by residual valences without actually entering the tooth structure. In vitro experiments, using powdered enamel, have conclusively demonstrated the possibility of the occurrence of this phenomenon (11, 12). Throughout the literature, we find a tendency on the part of investigators to neglect the differentiation between adsorption and actual penetration.

It is the purpose of this investigation to develop a method for studying tooth permeability under normal physiological conditions. For this purpose, sodium iodide was selected because it possesses properties which are ideal for this type of investigation: 1. It is not a normal constituent of teeth and hence its identification requires qualitative rather than quantitative methods. 2. It is of small molecular size comparable to the salts present in the saliva and hence bathing the teeth in vivo. It is especially comparable to fluoride salts. 3. It is excreted by the saliva (13) so that it is possible to maintain a constant solution of iodide externally contacting the teeth as well as introducing it systematically. 4. It presents the possibility of histological demonstration. 5. It is not toxic in low concentration and hence can be introduced in addition to the normal diet without causing any physiologic changes.

EXPERIMENTAL PROCEDURE

Two experimental dogs were used in this study. Prior to administration of the iodide, several teeth were extracted as controls. Full cast metal crowns were constructed for others and several teeth were ground on the buccal surface with a stone to remove the outer layer of enamel. Since it has been presumed that the outer layer of enamel is impermeable, (3), we wished to observe the effect of its removal. The remaining teeth were left intact and will be referred to as the experimental teeth.

The sodium iodide was administered, in addition to the usual diet, in 1 gm. gelatin capsules once a day for 30 days by inserting into the dogs' throat. At the end of this period the dogs were sacrificed and the teeth removed for histological and biochemical examination.

HISTOLOGICAL STUDY

An upper canine from one experimental dog, a lower premolar from another, and a control tooth from a dog which had never been fed sodium iodide were sectioned longitudinally and ground under oil to the thickness of 150 micra and examined microscopically. The teeth of the experimental animal differed

from that of the control animal only by exposure to sodium iodide. The sections were then covered with two drops of saturated solution of lead nitrate which precipitates an iodide as yellow lead iodide. Other sections were also examined, by using a saturated solution of mercuric bichloride which would produce an orange precipitate of mercuric iodide.

The section of the tooth from the dog given sodium iodide showed a light yellow precipitate, probably lead iodide, impregnating the dentin at the periphery of the pulp when lead nitrate had been applied. Under high magnification, it was possible to discern small yellow granules in some of the tubuli emanating from this layer. These may have been granules of lead iodide. The yellow stain produced was not sufficiently differentiated from the background color of the specimen to enable photographs to be taken. The control specimens gave no such color reaction.

The sections which were taken from the second experimental animal were stained with mercury bichloride, which produces an orange precipitate of mercuric iodide. No color change could be seen. The reaction between mercury and iodide is much less sensitive than that between lead and iodide. The lack of visible color change is probably due to the insufficient concentration of iodide to be visibly precipitated.

BIOCHEMICAL ANALYSIS

The teeth were mechanically cleaned and allowed to dry for a period of 1 week. At the end of this time they were cut at the cervical line with carborundum discs and the roots were discarded. The dentin was separated from the enamel mechanically by using a steel bur set up in a dental engine. Grinding was continued until the consistency of the tooth in contact with the bur and the color of the remaining portion indicated that the dentin had been removed, leaving a shell of enamel. The enamel shells were pulverized in a swedger until the particles passed through a 60 mesh sieve.

In order to insure the complete removal of dentin from the enamel, separation by the flotation method of Manley and Hodge (14) was attempted; however, the only sample large enough to be separated in this manner was that of the enamel of the experimental teeth. The remaining specimens were tested on the basis of mechanical separation. The tests for water soluble iodide were performed on the following samples: 1. Enamel and dentin from the control tooth. 2. Enamel and dentin from the experimental teeth. 3. Enamel and dentin from the ground experimental teeth. 4. Enamel and dentin from crowned experimental teeth. A blank of distilled water was run to test the purity of the reagents.

Twenty-five mg. samples of the above were placed in test tubes, each containing 10 cc. of distilled water, and left to stand for a week. They were shaken at frequent intervals to permit all the water soluble iodide to go into solution. The supernatent liquid was drained off at the end of 1 week, evaporated to a volume of several drops in a porcelain crucible, and tested in the following manner:

A few drops of bromine water were added to the test solution which was then

heated in order to oxidize the iodide to iodate. Several crystals of phenol were added to remove the excess bromine as tri-bromo-phenol. The solution was acidified with 2N sulfuric acid and several drops of fresh 5% potassium iodide were added. This converted the iodate to free iodine. One drop of starch solution was added; a blue color denoted the presence of iodine. According to Feigl (15), this test will produce a positive reaction with 0.1 gamma of iodine in a concentration of 1:500,000.

In addition to demonstrating the presence of iodine, the colors produced could be compared to give some idea of the relative concentration of the iodide in the various samples.

Results:

Samples	*Enamel*	*Dentin*
1. Extracted teeth	Colorless	Colorless
2. Experimental teeth	2 plus, 2 plus	5 plus, 6 plus
3. Crowned teeth	1 plus	4 plus, 4 plus
4. Ground teeth	6 plus, 8 plus	4 plus, 8 plus

SUMMARY OF RESULTS

1. Sodium iodide, a substance foreign to the normal dog's tooth when introduced in high concentration into the systemic circulation and saliva, was deposited in both the enamel and the dentin.

2. It was deposited to a much greater extent in the dentin than in the enamel.

3. Histological examination indicates a concentration of the iodide in the dentin layer approximating the pulp.

4. Grinding the surface of the enamel prior to the introduction of the iodide caused an increase in the deposition of sodium iodide in the enamel.

5. Crowning the teeth prior to the introduction of the iodide caused a decrease in the deposition of iodide in the enamel.

DISCUSSION

In discussing the results of this experiment, two factors must be kept in mind: 1. From the radioactive isotope studies it is apparent that only a minute amount of available metabolite finds its way into the teeth (7). 2. The biochemical tests were made on water soluble iodides. This means that any iodide adsorbed on the surface or combined with calcium salts would not go into solution and hence would not affect the test.

Since so little iodide finds its way into the teeth, the failure to stain this material throughout the dentin and enamel is no indication of its total absence, but rather of its absence in quantities large enough to be visibly precipitated by nitrate or mercuric chloride. On the other hand, the appearance of a visible precipitate in the innermost layer of dentin is evidence of a high concentration of iodide in this portion of the tooth. This is to be expected when we consider that the odontoblasts line the pulp and are in close proximity to the pulpal blood and lymph vessels.

Although the theory of the diffusion of dental lymph (tissue fluid) is generally

accepted, little is known of the nature of this fluid or the manner in which it is formed. Since our biochemical tests were made on water soluble iodide, it may be assumed that the iodide which gave the positive reaction was present in the pathways of the enamel and dentin and was carried there by this fluid. When we realize that the iodide salt is not a constituent of teeth, although it is present in the blood to a slight extent, (12 gamma per 100 cc.), it may be that when a certain concentration gradient for a substance is reached in the blood, it spills over into the dental lymph. This possibility is supported by the work of Howe (18) on lead poisoning. In advanced stages of plumbism, when the lead concentration of the blood is high, lead is visibly precipitated in the dentin and enamel near the dentino-enamel junction.

The transfer of substances from the blood to the dental lymph is probably accomplished by the odontoblasts. Secretory function of odontoblasts is further substantiated by Beams and King (16) who demonstrated changes in the Golgi apparatus of odontoblasts. According to Severinghaus (17), these changes are associated with secretory activity of the cell.

The amount of iodide found in the dentin was much greater than that found in the enamel. This is in agreement with the radioactive isotope studies and can be explained on the basis of the proximity of the dentin to the pulpal vessels and the existence of tubules in the dentin through which substances can diffuse.

When the enamel surface is ground, prior to the feeding of sodium iodide, it is found that considerably more iodide entered the enamel. Since the relationship of the dentin and pulp remains the same, the probable explanation is that breaking the continuity of the enamel surface makes it penetrable to the saliva.

Crowning of the teeth produced a slight decrease in the deposition of iodide in the enamel. This cannot, however, be considered a significant difference on the basis of our experimental procedure, since the color change from 2 plus to 1 plus may fall within the range of experimental error. In addition, there is the possibility that the crowns were not sufficiently adapted at the gingival margin to completely prevent the entrance of iodide from the saliva. With these factors in mind at this time it is impossible to draw any conclusions as to a differentiation between deposition of iodide through contact with the saliva and deposition via the systemic circulation. With further development of this method and more refined technique, it is probable that a definite conclusion can be reached.

SUMMARY AND CONCLUSIONS

The feeding of sodium iodide in large quantities results in its deposition in the teeth of dogs, to a greater extent in the dentin than in the enamel. It appears to be concentrated in the innermost layer of the dentin.

Grinding the enamel surface prior to feeding seems to render the tooth more permeable to the saliva.

No conclusions can be drawn as the effect of crowning the teeth.

The results of this experiment are open to criticism on the basis of the lack of a sufficient number of experimental animals to fully substantiate our conclusions. The purpose of the experiment, however, was to develop a method of study.

The findings are presented therefore primarily as indicative of the efficacy of the method.

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VARIABILITY OF THE PAIN THRESHOLD IN MAN[1]

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In 1940, Hardy, Wolff and Goodell (1) introduced a new method for determining the pain threshold in man, and applied the method in a study of the analgesic efficiency of drugs. They used infrared stimulation of the skin of the forehead and measured the intensity at the threshold with a sensitive radiometer. The pain thresholds they found were remarkably uniform from person to person and constant in each individual. All their observations fell within $\pm 12\%$ of the mean. Later, in a much larger series Schumacher, Goodell, Hardy, and Wolff (2) found a mean pain threshold slightly lower than their previous figure but showing the same uniformity and small standard deviation.

Slaughter and Wright (3), using a modification of the Hardy technique, failed to confirm these results. Their mean threshold values for each person, measured in degrees centigrade, when recalculated to take into account the initial forehead temperature ($34.0 \pm 0.5°$ C. [1]), showed a range of -20.8% to $+9.1\%$ of the mean. Lanier (4), using electrical stimulation, found a wide range of pain thresholds from -80% to $+300\%$ of the mean. His relative variability was 0.56. In only one-half his subjects could Lanier find good correlation between thresholds determined on 2 successive days, the other persons showing such poor correlation that the correlation coefficient for the entire group was $+0.55$.

Using the technique of Hardy, Wolff, and Goodell, a series of pain threshold determinations were made in this department with the object of determining the value and limitations of this method. As the further application of the method could not be made by us, it is hoped that the results obtained may be of interest and value to others.

METHOD

The subjects used in this investigation were young adult males (college students) and the authors. The tests were conducted in the evening, beginning about 7 P.M., the object being to complete a 4-hour test period.

The apparatus for stimulation of the persons was, as nearly as possible, a duplicate of that of Hardy, Wolff and Goodell (1). Light from a fan-cooled 1000 watt bulb was refracted into a beam of parallel rays which passed through holes in 2 shields. The first shield, revolving at 10 r.p.m., was circular with a semicircular slot which allowed the light to shine through for 3 seconds per revolution.

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The second shield, which was stationary, had a square hole, 2 centimeters on each side, covered by a flap which was raised and lowered by the operator during the period that the light was blocked by the first shield. The person was seated at the end of the optical bench with his head supported so that his forehead was 5 mm. beyond the second shield. With this arrangement, an area of 4 cm.^{2} of the subject's blackened forehead was illuminated for 3 seconds per exposure, the intensity of the light and the interval between exposures being controlled by the operator.

The radiometer consisted of 168 welded, blackened, iron-constantan junctions enclosed in a black box having a square aperture of 4 cm.^{2} in area, behind which the 84 hot junctions were distributed uniformly. After each determination, the radiometer was swung into the position formerly occupied by the person's forehead. The circular shield was stopped, the hot junctions were exposed to the beam of light, and measurements were made. The Coleman potentiometer showed that, in the range in which the pain thresholds lay, roughly 1 additional millivolt was set up in this radiometer for each volt applied across the filament of the lamp.

A description of the pain sensation as differentiated from the heat sensation, taken directly from Hardy, Wolff and Goodell (1) enabled each of us to distinguish the pain sensation readily. Test exposures on the persons were made at 30 second intervals until the threshold was determined. In case the stimulation was submaximal, 60 seconds were allowed to elapse between exposures. Sittings were spaced at 20 minute intervals, thus permitting 3 determinations per person per hour.

RESULTS

The present report is concerned with the pain thresholds determined in 16 individuals. From 2 to 6 threshold determinations per evening were made on each subject at about 1 to 3 week intervals for periods up to 1 year. An average of 3.1 determinations were made on each person each evening. The average number of evenings per subject was 7.25, with a range of 2–16. In all, 327 pain threshold determinations were made. The mean pain threshold was 67.7 millivolts with a standard deviation of ± 23.5 millivolts and a coefficient of variability of 0.34. Only 64.5% of the threshold determinations fell within 1 standard deviation of the mean. This variability in pain threshold is similar to that reported by Slaughter and Wright (3) and Lanier (4), the latter finding a standard deviation equal to about one-half the mean.

Upon examination of the series of determinations on each subject, it was found that there was no real correlation between the mean threshold of one evening and that of an evening a week later. Instead of this, it was found that 6 of the 9 subjects who were tested on 5 or more evenings showed a gradual, though inconstant, rise in pain threshold over a long period of time. *Fig. 1* shows the rise in threshold of 2 persons as well as the variations in the threshold of a person whose pain threshold was relatively more constant. Table I shows the mean

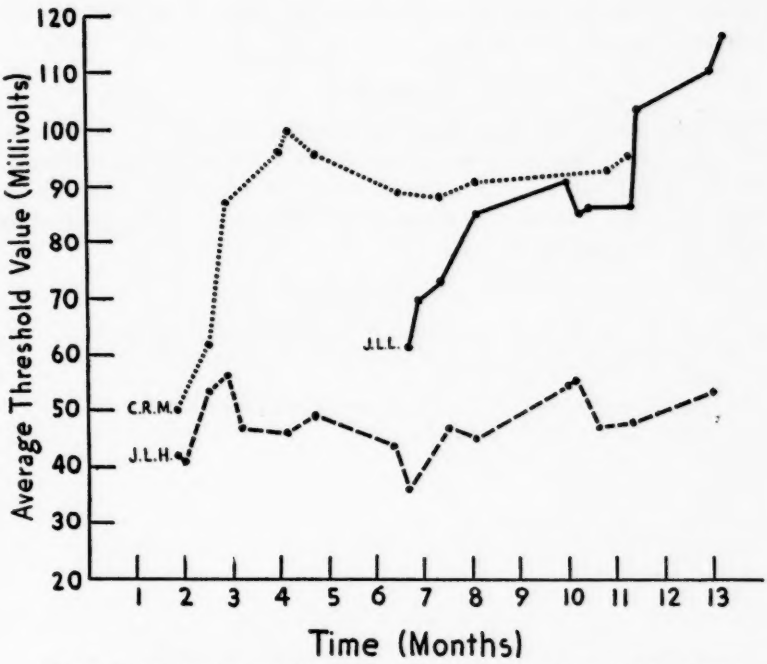

Fig. 1. Variations in pain thresholds of 3 persons over long periods of time

TABLE I

Variability of the pain threshold in 16 subjects

SUBJECT	MEAN P.T. IN MV.	RANGE IN MV.	NO. THRESHOLD DETERMINATIONS	NO. EVENINGS
B. S. A.	46.3	40.0– 51.0	6	2
C. M. B.	43.9	32.1– 63.8	9	3
D. B.	37.0	31.2– 42.5	7	2
R. C. C.	49.5	42.0– 59.1	17	4
J. L. H.	47.4	36.9– 63.1	45	16
M. L. H.	81.5	74.2– 90.7	10	2
J. L. L.	88.0	60.0–118.6	30	11
H. M. L.	76.2	61.1–100.4	9*	3
A. M. M.	48.4	35.1– 59.2	16	5
C. R. M.	86.0	44.1–101.6	34	11
B. H. N.	46.9	36.4– 64.8	23	5
R. J. P.	52.6	16.6–112.9	50	13
H. C. S.	88.0	70.4–117.0	24	8
H. M. S.	85.7	72.0– 98.9	20*	7
J. R. S.	65.0	61.1– 73.5	10*	4
J. R. Sp.	77.8	44.4– 99.5	17	5

* 7 previous determinations made using a different radiometer.

pain threshold and the range for each person and also the number of evenings and determinations in each series.

SUMMARY

A significant variability was found in the pain threshold determinations in different persons and also in the same person over a period of time. The average pain threshold of 16 young adults was 67.7 millivolts with a standard deviation of ±23.5 millivolts. About two-thirds of the persons showed a gradual rise in threshold over a period of months.

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